Supplementary Information

Insights into Plant Cell Wall Structure, Architecture and Integrity using Glycome Profiling of Native and AFEX[TM] Pretreated Biomass

Sivakumar Pattathil*[1,2], Michael G. Hahn[1,2], Bruce E. Dale[3], Shishir P. S. Chundawat*[3,4]

Sivakumar Pattathil

Email : siva@ccrc.uga.edu

Phone : 706 542 4451

Fax   : 706 542 4412

Michael G. Hahn

Email: hahn@ccrc.uga.edu

Bruce E. Dale

bdale@egr.msu.edu

Shishir P. S. Chundawat

shishir.chundawat@rutgers.edu

Phone : 848-445-3678

Fax   : 517-336-4615

[1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602, USA

[2]BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

[3]DOE Great Lakes Bioenergy Research Center, Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI 48824, USA

[4]Current Address: Department of Chemical and Biochemical Engineering, C-150A Engineering Building, Rutgers The State University of New Jersey, 98 Brett Road, Piscataway, NJ 08854

*Corresponding authors

Supplementary Figure Legends

Supplementary Figure S1: Heat map analyses of the relative abundance of major non-cellulosic cell wall glycan epitopes in 4M KOH extracts from eight phylogenetically diverse plant biomasses with or without AFEX™ pretreatment. 4M KOH extracts were prepared from cell walls isolated from diverse classes of plant biomass as explained in Materials and Methods. The extracts were subsequently screened by ELISA using a comprehensive suite of cell wall glycan directed mAbs. Binding response values are depicted as heat maps with a black-red-bright yellow color scheme, where bright yellow represents strongest binding and black no binding. The dotted boxes outline sets of antibodies whose binding signals were used for the scatter plot analyses shown in Fig. S2. The amount of carbohydrate material recovered per gram of cell wall is depicted in the bar graphs (purple) above the heat maps. The panel on the right hand side of the heat map shows the groups of mAbs based on the class of cell wall glycan they each recognize.

Supplementary Figure S2: Scatter plot analyses of the relative abundance of major non-cellulosic cell wall glycan epitopes in 4M KOH extracts from eight phylogenetically diverse plant biomasses with or without AFEX™ pretreatment. 4M KOH extracts were prepared from cell walls isolated from diverse classes of plant biomass as explained in Materials and Methods. The extracts were subsequently screened by ELISA using a comprehensive suite of cell wall glycan directed mAbs. Comparisons of the relative abundances of epitopes characteristic of three cell wall polysaccharide classes, xyloglucans (blue dots), xylans (red dots) and pectin/arabinogalactans (green dots) in the 4M KOH extracts before and after medium severity AFEX™ pretreatment of diverse plant biomass samples (See Figure 2 for more details). Data are re-plotted from Figure S1, but are normalized to represent mAb binding strength per mass of original cell wall. The red dashed lines denote the expected position if the abundance of these glycan epitopes was unchanged after AFEX™ pretreatment. Data points above and below the dashed lines represent increased or decreased glycan epitope abundance, respectively, after AFEX™ pretreatment. Note that the y-axis scales are different for individual plots to permit visualization of trends and magnitudes of normalized epitope abundances.

Supplementary Figure S3: Heat map analyses of the relative abundance of major non-cellulosic cell wall glycan epitopes in chlorite extracts from eight phylogenetically diverse plant biomasses with or without AFEX™ pretreatment. Chlorite extracts were prepared from cell walls isolated from diverse classes of plant biomass as explained in Materials and Methods. The extracts were subsequently screened by ELISA using a comprehensive suite of cell wall glycan directed mAbs. Binding response values are depicted as heat maps with a black-red-bright yellow color scheme, where bright yellow represents strongest binding and black no binding. The dotted boxes outline sets of antibodies whose binding signals were used for the scatter plot analyses shown in Fig. S4. The amount of carbohydrate material recovered per gram of cell wall is depicted in the bar graphs (purple) above the heat maps. The panel on the right hand side of the heat map shows the groups of mAbs based on the class of cell wall glycan they each recognize.

Supplementary Figure S4: Scatter plot analyses of the relative abundance of major non-cellulosic cell wall glycan epitopes in chlorite extracts from eight phylogenetically diverse plant biomasses with or without AFEX™ pretreatment. Chlorite extracts were prepared from cell walls isolated from diverse classes of plant biomass as explained in Methods. The extracts were subsequently screened by ELISA using a comprehensive suite of cell wall glycan directed mAbs. Comparisons of the relative abundances of epitopes characteristic of three cell wall polysaccharide classes, xyloglucans (blue dots), xylans (red dots) and pectin/arabinogalactans (green dots) in the chlorite extracts before and after medium severity AFEX™ pretreatment of diverse plant biomass samples (See Figure 2 for more details). Data are re-plotted from Figure S3, but are normalized to represent mAb binding strength per mass of original cell wall. The red dashed lines denote the expected position if the abundance of these glycan epitopes was unchanged after AFEX™ pretreatment. Data points above and below the dashed lines represent increased or decreased glycan epitope abundance, respectively, after AFEX™ pretreatment. Note that the y-axis scales are different for individual plots to permit visualization of trends and magnitudes of normalized epitope abundances.

Supplementary Figure S5: Heat map analyses of the relative abundance of major non-cellulosic cell wall glycan epitopes in 4M KOHPC extracts from eight phylogenetically diverse plant biomasses with or without AFEX™ pretreatment. 4M KOHPC extracts were prepared from cell walls isolated from diverse classes of plant biomass as explained in Materials and Methods. The extracts were subsequently screened by ELISA using a comprehensive suite of cell wall glycan directed mAbs. Binding response values are depicted as heat maps with a black-red-bright yellow color scheme, where bright yellow represents strongest binding and black no binding. The dotted boxes outline sets of antibodies whose binding signals were used for the scatter plot analyses shown in Fig. S6. The amount of carbohydrate material recovered per gram of cell wall is depicted in the bar graphs (purple) above the heat maps. The panel on the right hand side of the heat map shows the groups of mAbs based on the class of cell wall glycan they each recognize.

Supplementary Figure S6: Scatter plot analyses of the relative abundance of major non-cellulosic cell wall glycan epitopes in 4M KOHPC extracts from eight phylogenetically diverse plant biomasses with or without AFEX™ pretreatment. 4M KOHPC extracts were prepared from cell walls isolated from diverse classes of plant biomass as explained in Materials and Methods. The extracts were subsequently screened by ELISA using a comprehensive suite of cell wall glycan directed mAbs. Comparisons of the relative abundances of epitopes characteristic of three cell wall polysaccharide classes, xyloglucans (blue dots), xylans (red dots) and pectin/arabinogalactans (green dots) in the 4M KOHPC extracts before and after medium severity AFEX™ pretreatment of diverse plant biomass samples (See Figure 2 for more details). Data are re-plotted from Figure S5, but are normalized to represent mAb binding strength per mass of original cell wall. The red dashed lines denote the expected position if the abundance of these glycan epitopes was unchanged after AFEX™ pretreatment. Data points above and below the dashed lines represent increased or decreased glycan epitope abundance, respectively, after AFEX™

pretreatment. Note that the y-axis scales are different for individual plots to permit visualization of trends and magnitudes of normalized epitope abundances.

Supplementary Figure S7: Cellulose and neutral sugar compositions of the untreated plant biomasses used in this study. Error bars depict standard deviations of data from mean values reported for assays conducted in triplicate. Data plotted in the graph are also shown in the table below to allow comparison between low abundance components.

Supplementary Figure S8: Lignin content, based on the acetyl bromide method (A), and lignin composition, as S/G/H monomer units (B), of untreated plant biomasses used in this study. Error bars depict standard deviations of data from mean values reported for assays conducted in triplicate.
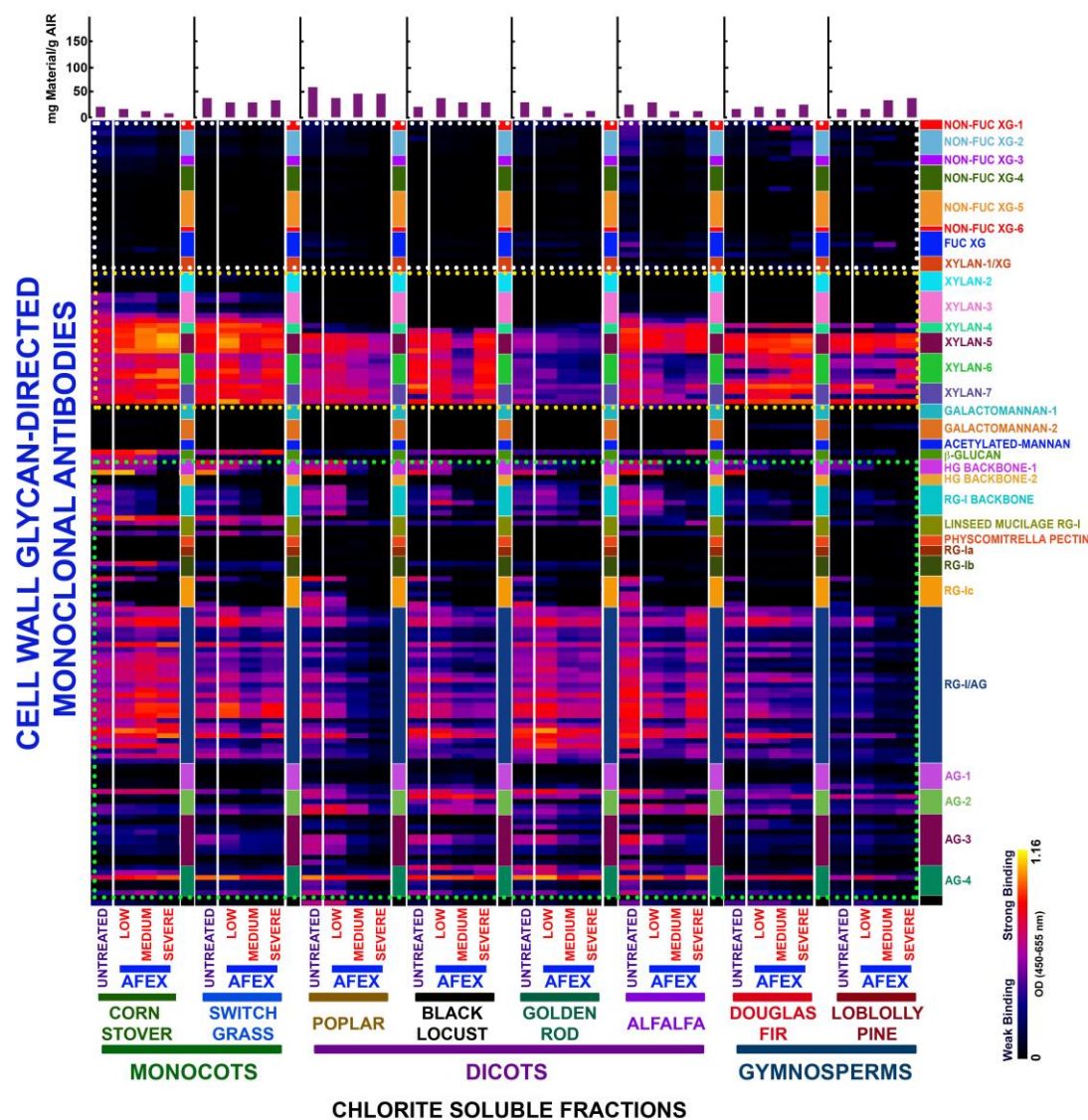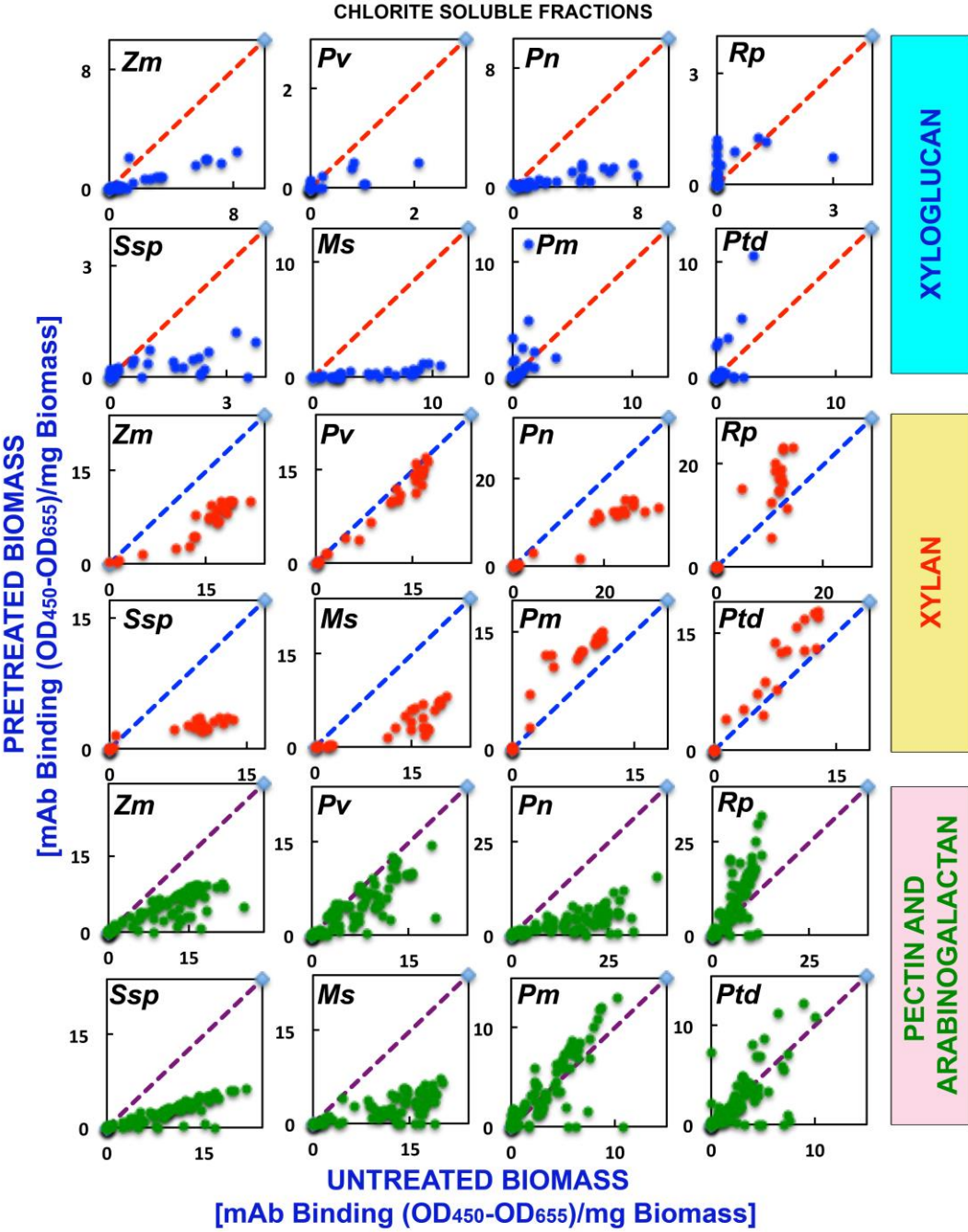
Supplementary Figure S1

Supplementary Figure S2

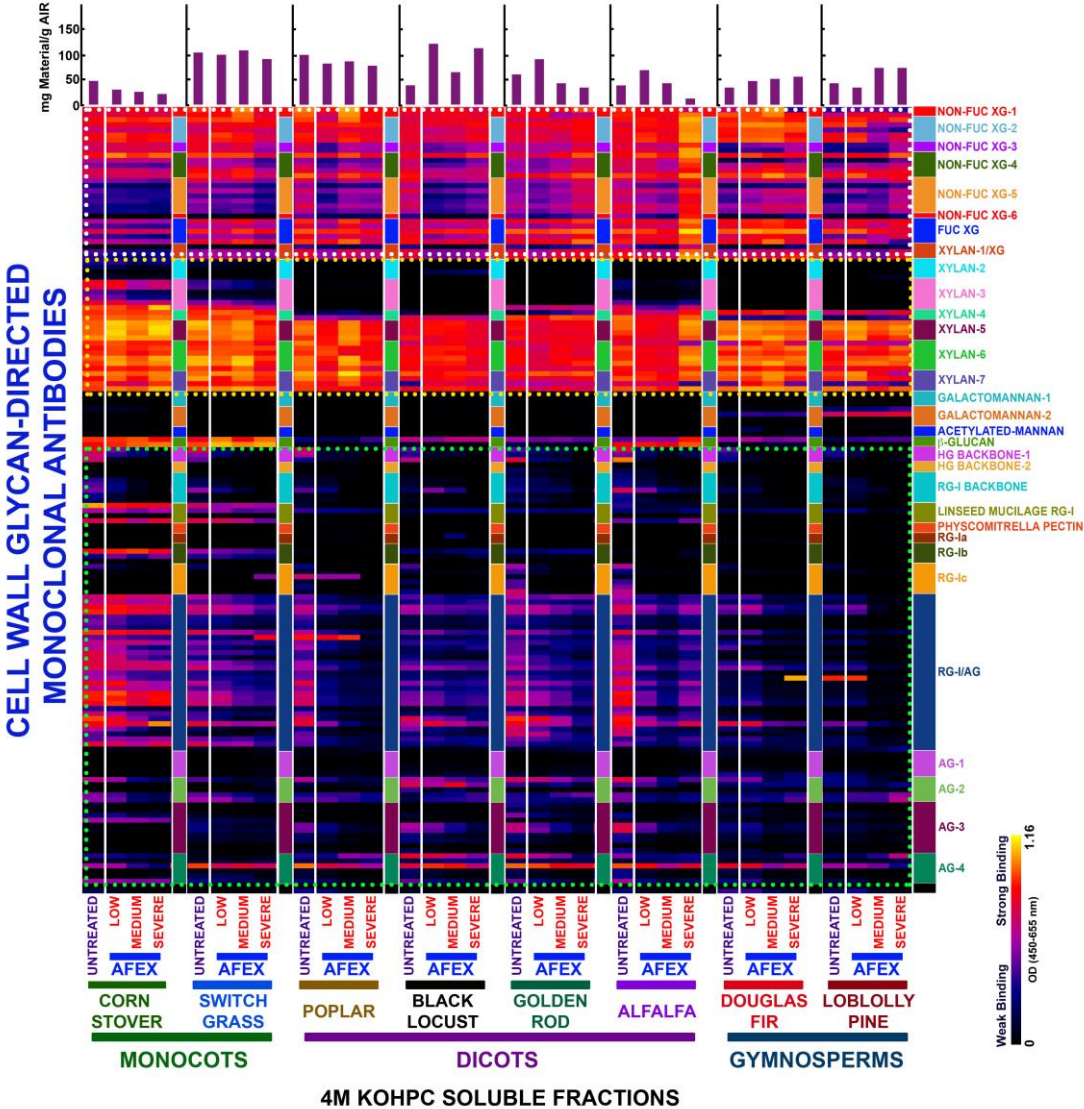4M KOH SOLUBLE FRACTIONS
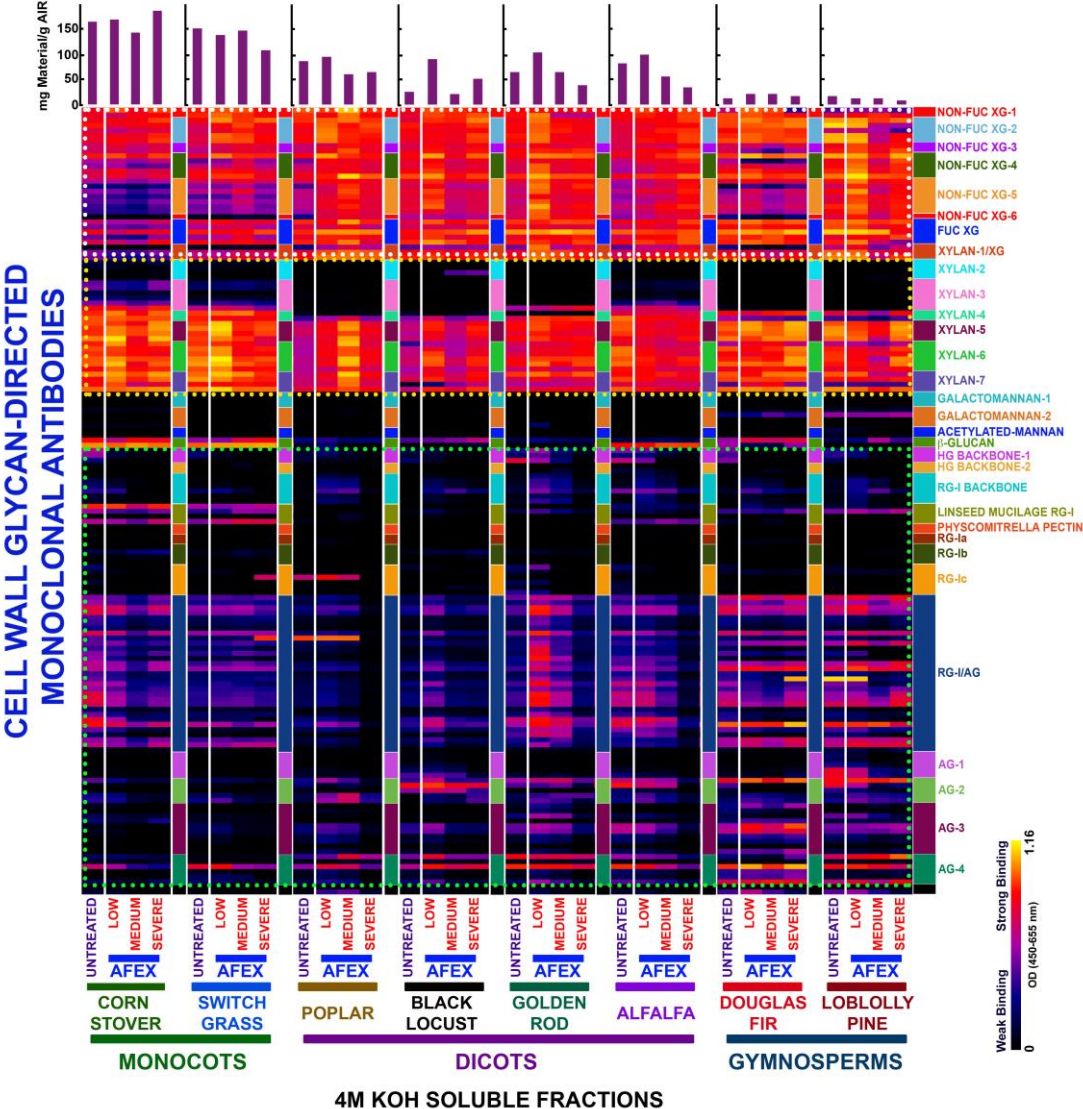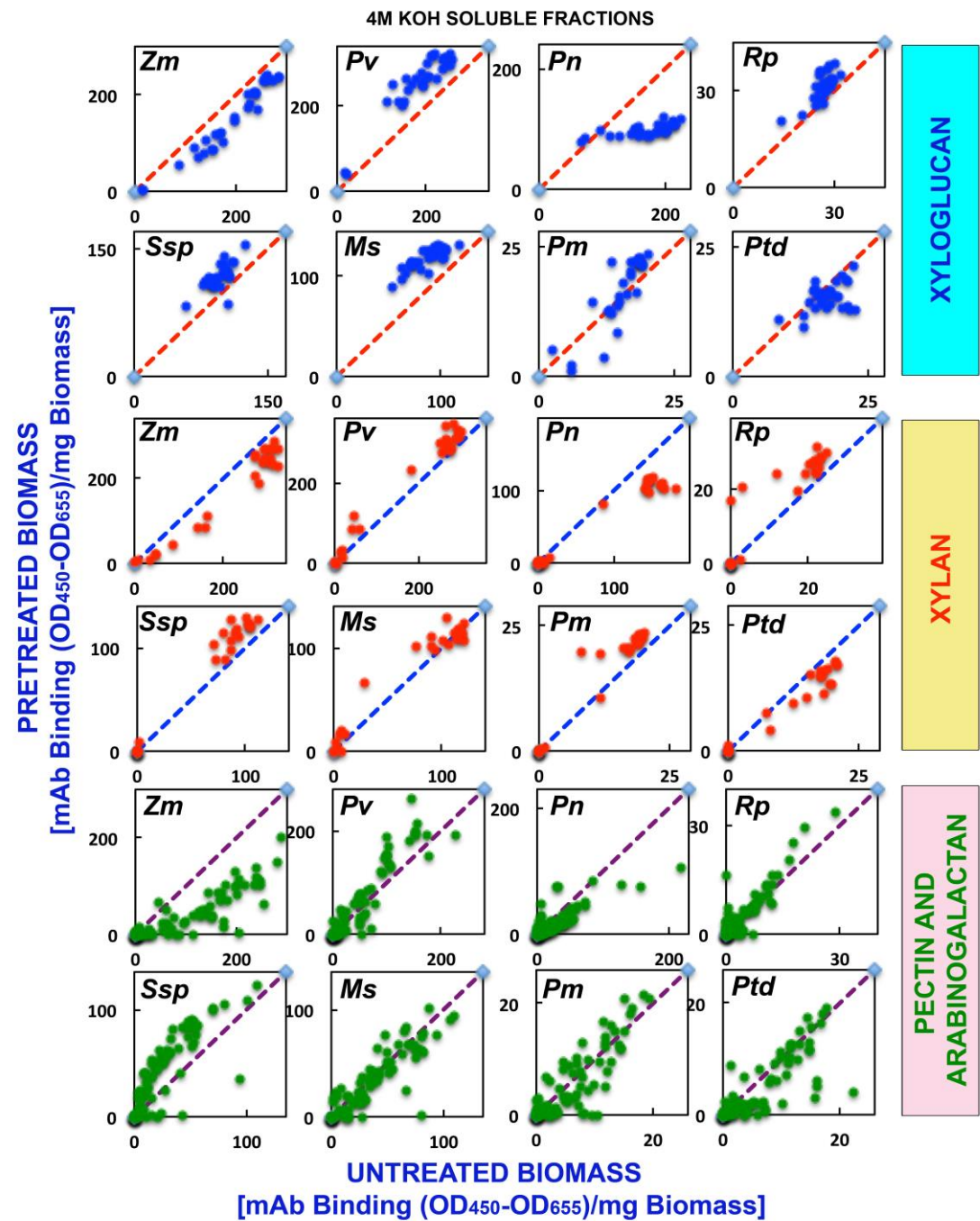
Supplementary Figure S3

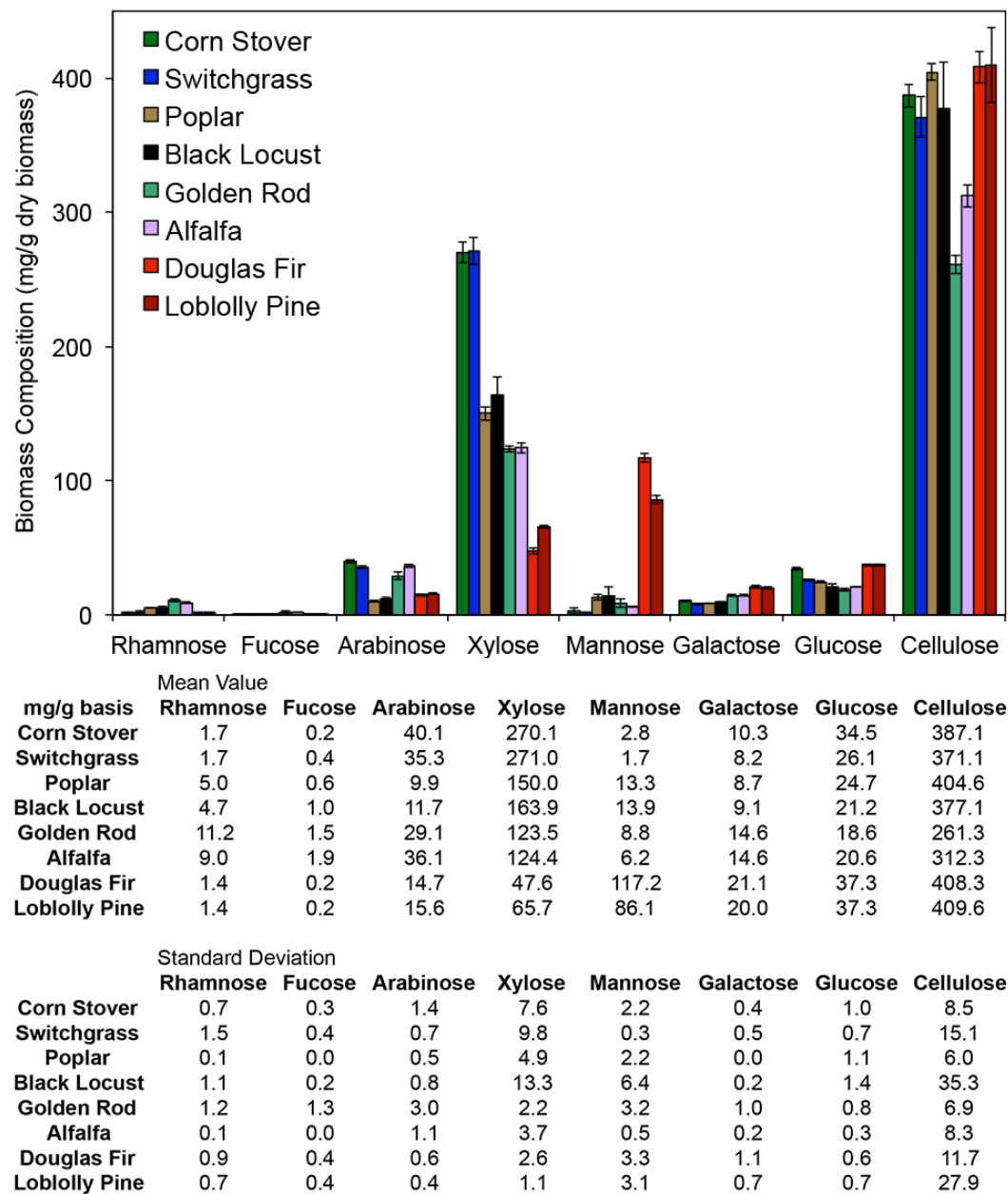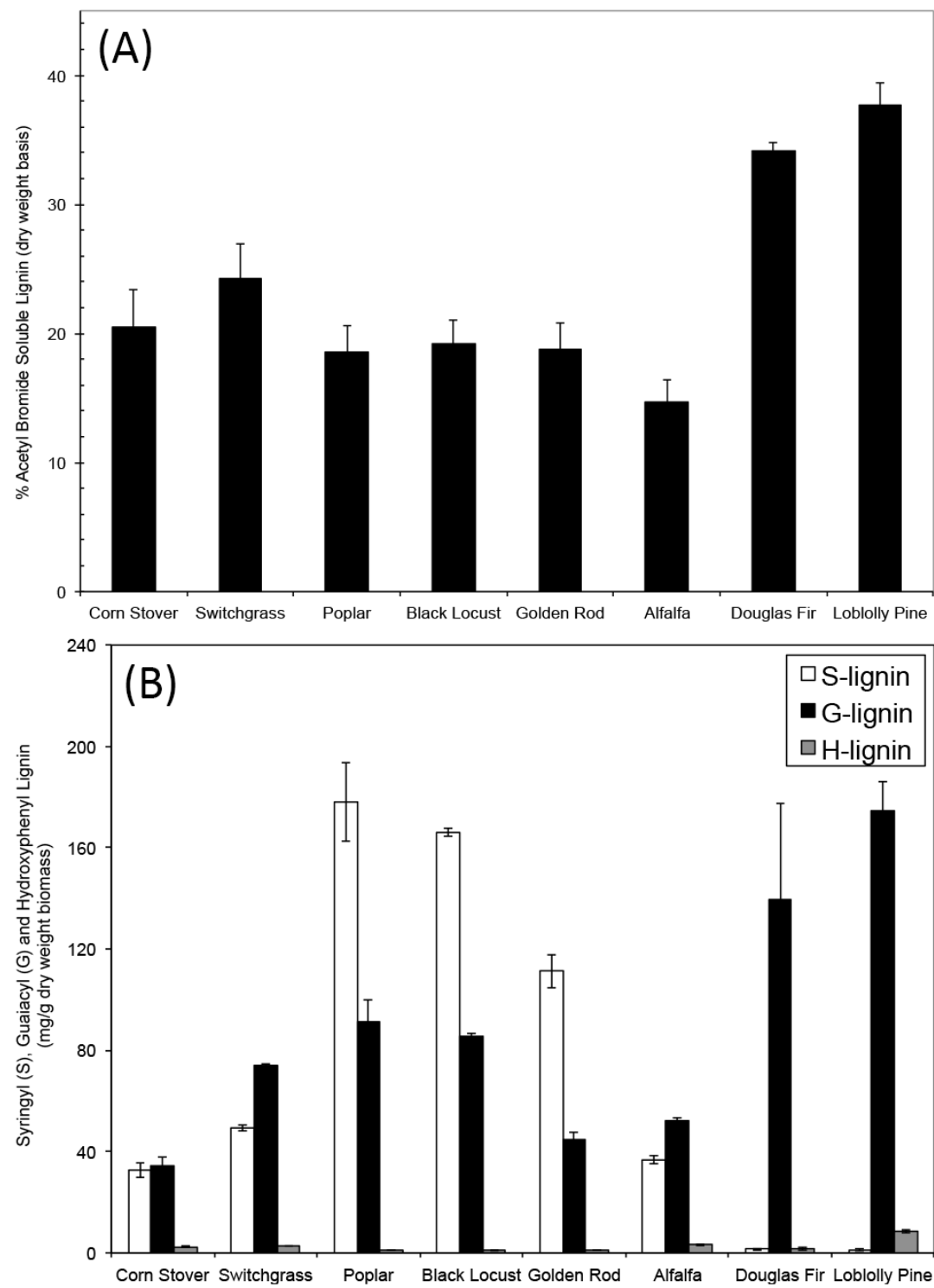**Supplementary Figure S4**

Supplementary Figure S5

Supplementary Figure S6

4M KOHPC SOLUBLE FRACTIONS

Supplementary Figure S7

| | Mean Value | | | | | | | |
mg/g basis	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Cellulose
Corn Stover	1.7	0.2	40.1	270.1	2.8	10.3	34.5	387.1
Switchgrass	1.7	0.4	35.3	271.0	1.7	8.2	26.1	371.1
Poplar	5.0	0.6	9.9	150.0	13.3	8.7	24.7	404.6
Black Locust	4.7	1.0	11.7	163.9	13.9	9.1	21.2	377.1
Golden Rod	11.2	1.5	29.1	123.5	8.8	14.6	18.6	261.3
Alfalfa	9.0	1.9	36.1	124.4	6.2	14.6	20.6	312.3
Douglas Fir	1.4	0.2	14.7	47.6	117.2	21.1	37.3	408.3
Loblolly Pine	1.4	0.2	15.6	65.7	86.1	20.0	37.3	409.6

| | Standard Deviation | | | | | | | |
	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Cellulose
Corn Stover	0.7	0.3	1.4	7.6	2.2	0.4	1.0	8.5
Switchgrass	1.5	0.4	0.7	9.8	0.3	0.5	0.7	15.1
Poplar	0.1	0.0	0.5	4.9	2.2	0.0	1.1	6.0
Black Locust	1.1	0.2	0.8	13.3	6.4	0.2	1.4	35.3
Golden Rod	1.2	1.3	3.0	2.2	3.2	1.0	0.8	6.9
Alfalfa	0.1	0.0	1.1	3.7	0.5	0.2	0.3	8.3
Douglas Fir	0.9	0.4	0.6	2.6	3.3	1.1	0.6	11.7
Loblolly Pine	0.7	0.4	0.4	1.1	3.1	0.7	0.7	27.9

Supplementary Figure S8

Supplementary Tables

Supplementary Table S1: Detailed list of cell wall glycan-directed monoclonal antibodies (mAbs) used for glycome profiling analyses. The groupings of antibodies are based on a hierarchical clustering of ELISA data generated from a screen of all mAbs against a comprehensive panel of plant polysaccharide preparations (Pattathil et al., 2010; Pattathil et al., 2012) that clusters mAbs according to the predominant polysaccharides that they recognize. The majority of listings link to the Wall*Mab*DB plant cell wall monoclonal antibody database (http://www.wallmabdb.net) that provides detailed descriptions of each mAb, including immunogen, antibody isotype, epitope structure (to the extent known), supplier information, and related literature citations.

Glycan Group Recognized   mAb Names

Glycan Group Recognized	mAb Names
Non-Fucosylated Xyloglucan-1	CCRC-M95 CCRC-M101
Non-Fucosylated Xyloglucan-2	CCRC-M104 CCRC-M89 CCRC-M93 CCRC-M87 CCRC-M88
Non-Fucosylated Xyloglucan-3	CCRC-M100 CCRC-M103
Non-Fucosylated Xyloglucan-4	CCRC-M58 CCRC-M86 CCRC-M55 CCRC-M52 CCRC-M99

Non-Fucosylated Xyloglucan-5	CCRC-M54 CCRC-M48 CCRC-M49 CCRC-M96 CCRC-M50 CCRC-M51 CCRC-M53
Non-Fucosylated Xyloglucan-6	CCRC-M57
Fucosylated Xyloglucan	CCRC-M102 CCRC-M39 CCRC-M106 CCRC-M84 CCRC-M1
Xylan-1/XG	CCRC-M111 CCRC-M108 CCRC-M109
Xylan-2	CCRC-M119 CCRC-M115 CCRC-M110 CCRC-M105
Xylan-3	CCRC-M117 CCRC-M113 CCRC-M120 CCRC-M118 CCRC-M116 CCRC-M114
Xylan-4	CCRC-M154 CCRC-M150

Xylan-5	CCRC-M144
	CCRC-M146
	CCRC-M145
	CCRC-M155
Xylan-6	CCRC-M153
	CCRC-M151
	CCRC-M148
	CCRC-M140
	CCRC-M139
	CCRC-M138
Xylan-7	CCRC-M160
	CCRC-M137
	CCRC-M152
	CCRC-M149
Galactomannan-1	CCRC-M75
	CCRC-M70
	CCRC-M74
Galactomannan-2	CCRC-M166
	CCRC-M168
	CCRC-M174
	CCRC-M175
Acetylated Mannan	CCRC-M169
	CCRC-M170
β-Glucan	LAMP
	BG1
HG Backbone-1	CCRC-M131
	CCRC-M38
	JIM5

HG Backbone-2	JIM136 JIM7
RG-I Backbone	CCRC-M69 CCRC-M35 CCRC-M36 CCRC-M14 CCRC-M129 CCRC-M72
Linseed Mucilage RG-I	JIM3 CCRC-M40 CCRC-M161 CCRC-M164
Physcomitrella Pectin	CCRC-M98 CCRC-M94
RG-Ia	CCRC-M5 CCRC-M2
RG-Ib	JIM137 JIM101 CCRC-M61 CCRC-M30
RG-Ic	CCRC-M23 CCRC-M17 CCRC-M19 CCRC-M18 CCRC-M56 CCRC-M16
RG-I/Arabinogalactan	CCRC-M60 CCRC-M41 CCRC-M80 CCRC-M79

	CCRC-M44
	CCRC-M33
	CCRC-M32
	CCRC-M13
	CCRC-M42
	CCRC-M24
	CCRC-M12
	CCRC-M7
	CCRC-M77
	CCRC-M25
	CCRC-M9
	CCRC-M128
	CCRC-M126
	CCRC-M134
	CCRC-M125
	CCRC-M123
	CCRC-M122
	CCRC-M121
	CCRC-M112
	CCRC-M21
	JIM131
	CCRC-M22
	JIM132
	JIM1
	CCRC-M15
	CCRC-M8
	JIM16
Arabinogalactan-1	JIM93
	JIM94
	JIM11
	MAC204
	JIM20
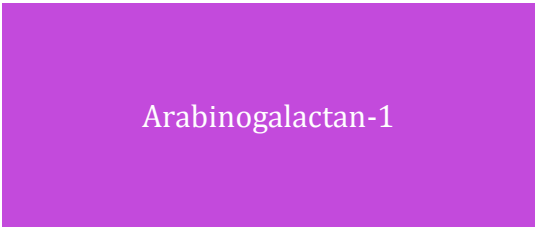
Arabinogalactan-2	JIM14
	JIM19
	JIM12
	CCRC-M133
	CCRC-M107
Arabinogalactan-3	JIM4
	CCRC-M31
	JIM17
	CCRC-M26
	JIM15
	JIM8
	CCRC-M85
	CCRC-M81
	MAC266
	PN 16.4B4
Arabinogalactan-4	MAC207
	JIM133
	JIM13
	CCRC-M92
	CCRC-M91
	CCRC-M78
Unidentified	MAC265
	CCRC-M97

Supplementary Table S2: Analyses of scatter plots generated from normalized mAb binding responses derived from glycome profiling data sets of 4M KOH, chlorite and 4M KOHPC extracts isolated from untreated and medium AFEX™ pretreated biomass (Figures, S2, S4 and S6). The table summarizes overall status in the abundance of epitopes recognized by XG, xylan and pectin/arabinogalactan mAbs in each extract from medium regime AFEX™ pretreated biomass as "reduced", "enhanced" or "no change" in comparison to the respective untreated biomass. Green and grey highlights denote similar and non-similar trends in related phylogenetic classes of plant biomass, respectively.

Biomass Type	Xyloglucan Epitope Abundance			Xylan Epitope Abundance			Pectin/AG Epitope Abundance		
	4M KOH	Chlorite	4M KOHPC	4M KOH	Chlorite	4M KOHPC	4M KOH	Chlorite	4M KOHPC
Overall scatterplot trends exhibited by AFEX™ pretreated Angiosperm Monocot Grasses									
Corn Stover	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced
Switchgrass	Enhanced	Reduced	No change	Enhanced	No change	No change	No change	Reduced	Reduced
Overall scatterplot trends exhibited by AFEX™ pretreated Woody Angiosperm Dicots									
Poplar	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	No change	Reduced	Reduced
Black Locust	Enhanced	Enhanced	No change	Enhanced	Enhanced	Enhanced	No change	Enhanced	No change
Overall scatterplot trends exhibited by AFEX™ pretreated Herbaceous Angiosperm Dicots									
Golden Rod	Enhanced	Reduced	Reduced	Enhanced	Reduced	Reduced	Enhanced	Reduced	Reduced
Alfalfa	Enhanced	Reduced	Reduced	No change	Reduced	Reduced	No change	Reduced	Reduced
Overall scatterplot trends exhibited by AFEX™ pretreated Woody Gymnosperms									
Douglas Fir	No change	No change	Enhanced	No change	Enhanced	Enhanced	No change	No change	No change
Loblolly Pine	No change	No change	Enhanced	No change	Enhanced	Enhanced	No change	No change	No change

References:

Pattathil S, Avci U, Baldwin D, *et al.* 2010. A comprehensive toolkit of plant cell wall glycan-directed monoclonal antibodies. *Plant Physiology* **153,** 514-525.

Pattathil S, Avci U, Miller JS, Hahn MG. 2012. Immunological approaches to plant cell wall and biomass characterization: Glycome profiling. In: Himmel M (ed) Biomass Conversion: Methods and Protocols. Springer Science + Business Media, LLC, New York, NY, pp 61-72.